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CERTIFICATE OF VERIFICATION

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Federal Republic of Germany

state that the attached document is a true and complete translation to the best of my knowledge of the German Patent Application No. DE 19846838 A1.

Dated this twenty-ninth day of January 2007

Signature of translator:____________________

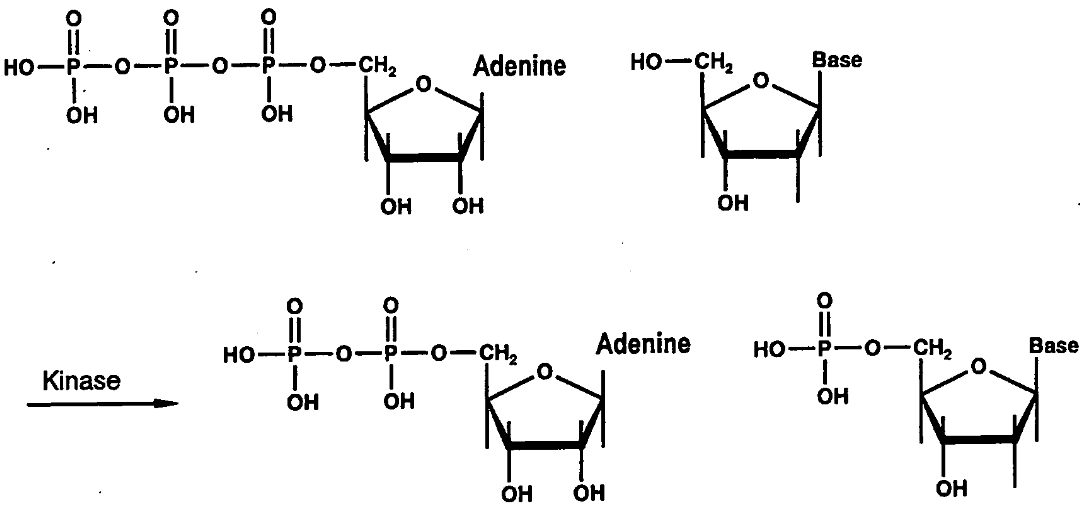
Sabine F.K. Town

Description

The present invention concerns a recombinant kinase from insect cells such as Drosophila melanogaster which remains stable during nucleoside monophosphate synthesis without the addition of stabilizing SH reagents, without stabilizing proteins and without detergents and which accepts all four natural deoxynucleosides. Furthermore, the present invention concerns a DNA sequence encoding the kinase according to the invention as well as a process for producing the kinase according to the invention and its use during nucleoside monophosphate synthesis.

(Deoxy)-nucleoside kinases catalyze the phosphorylation of nucleosides or deoxynucleosides to form the corresponding nucleotide monophosphates and therefore play an important role in the "salvage pathway" of nucleotide metabolism.

The catalyzed reaction is:



Deoxynucleoside monophosphates are in turn starting products for deoxynucleoside triphosphates which are being used to an increasing extent as reagents for the PCR reaction.

Deoxynucleoside monophosphates can at present be obtained by three methods:

1. from the hydrolysis of fish sperm
2. by chemical synthesis from deoxynucleosides
3. by enzymatic synthesis from deoxynucleosides.

The previously known methods have a number of disadvantages. Thus, during the hydrolysis of fish sperm all 4 monophosphates are produced in about the same proportions which is a fact that is at odds with the requirements of the market (for example d-UTP that is in some cases used instead of d-TTP, is prepared from d-CTP). In addition, the d-TTP that is formed during hydrolysis is contaminated with approximately 2 % d-UTP and is almost impossible to isolate.

Furthermore, the animal origin of the educts has to be judged to be critical from a regulatory point of view (GMP). Moreover, the market for monophosphates from fish sperm is very limited.

A large number of byproducts are formed during the chemical synthesis which are difficult to separate by chromatographic purification. In addition, several bases (e.g. guanosine) have to be provided with protective groups before phosphorylation which considerably complicates the synthesis.

The disadvantages of the state of the art were overcome by providing a recombinant multifunctional deoxynucleoside kinase from insect cells such as in particular Drosophila melanogaster (Dm-dNK) which remains stable during nucleoside monophosphate synthesis without the addition of stabilizing SH reagents, and without stabilizing proteins and detergents and which accepts all four natural deoxynucleosides: thymidine (dThd), deoxycytidine (dCyd), deoxyadenosine (dAdo) and deoxyguanosine (dGuo). In the sense of the present invention stable means that there is practically no decrease in the conversion rate of the catalyzed reaction within 5 hours, preferably within 10 hours and particularly preferably within 12 hours at 37°C. It is surprising that the enzyme remains stable for such a long time without addition of stabilizers containing thiol. This stability has so far not been observed with other kinases (1-9). The omission of these stabilizers when using the kinase according to the invention for the synthesis reduces the costs of the synthesis and, above all, greatly simplifies the purification of the products.

Furthermore, previously known kinases have a considerably higher substrate specificity. As a consequence a specific kinase is no longer needed in each case to synthesize the individual nucleosides. The low specificity is particularly advantageous for the synthesis of modified nucleoside analogues, such as dideoxynucleosides or base-modified or sugar-modified nucleosides. Base-modified nucleosides are for example 7-deaza-nucleosides, C-nucleosides and nucleotides whose base is labelled with reporter groups (dyes, digoxigenin, biotin). Sugar-modified nucleosides are for example azathymidine, arabinosyl-thymidine. The kinetic constants of the Drosophila kinase compared to known analogous enzymes are listed in table 1. The specific activity kc of the kinase according to the invention is several times higher than that of the previously known kinases. Thus, a considerably lower amount of enzyme is required to synthesize dNMPs. (factor 3.5 – 14000, cf. Kc values in table 1). The specificity constant (k_c/K_M) of the kinase according to the invention exceeds that of the hitherto known kinases by several powers and is of the order of magnitude of the diffusion constant. This leads to a complete conversion when the kinase is used for d-NMP synthesis. They are 2-6500-fold higher than the previously known kinases, see Fig. 1.

Surprisingly, the enzyme according to the invention is still stable at 60°C which is also advantageous for the reaction procedure.

The present invention additionally concerns kinases from other animal species of the Hexapoda class which have comparable properties to those of the Drosophila kinase. Especially those kinases which have essentially the stability and substrate specificity described above. Preferred kinases are those isolated from the subclass of Pterygota, particularly preferably from the order Diptera, and particularly preferably from the Drosophilidae family. A further subject matter of the invention is a DNA sequence which codes for the kinase according to the invention. The DNA sequence according to the invention is characterized in that the primers listed in the following hybridize to the DNA sequence of the kinase according to the invention:

SEQ ID No.: 2
GGGAAGTGGCAGGAGTAGCTCCCG

SEQ ID No.: 3
CTCCCGTTGTAGCCGTCGCCCTTCTGG

SEQ ID No.: 4
GACGACTGGCTCGGGCAGCTCTTCACCGCGTTG

SEQ ID No.: 5
TTCGATTTTTATTACCTCGCGAGGTAA

SEQ ID No.: 6
AGGTAAAAATCGCGAGCGATAACGAAGCAC

SEQ ID No.: 7
CACCGCATGCTTGCGTAGGCCGTCGCCCGAGCAAGACTCCTC

SEQ ID No.: 8
GACTACATGTTTCTAGGGTTCTTCACC

The present invention also concerns those kinases and DNA sequences to the DNA sequence of which oligonucleotides having the SEQ ID No.: 2, 3, 5, 7 and 8 or having the SEQ ID No.: 2, 4, 5, 7 and 8 or having the SEQ ID No.: 2, 5, 6, 7 and 8 hybridize.

The following hybridization conditions are advantageous in this connection:

- Hybridization: 0.75 M NaCl, 0.15 Tris, 10 mM EDTA, 0.1 % sodium pyrophosphate, 0.1 % SLS, 0.03 % BSA, 0.03 % Ficoll 400, 0.03 % PVP and 100 µg/ml boiled calf thymus DNA at 50°C for approximately 12 hours.

- Washing: 3 x 30 minutes with 0.1 X SET, 0.1 % SDS, 0.1 % sodium pyrophosphate and 0.1 M phosphate buffer at 45°C.

The kinase sequence according to the invention is shown in Fig. 5, SEQ ID No.: 1.

The DNA sequence according to the invention can be obtained from Drosophila melanogaster using the process described in the following:

A pBluescript SK +/- phagmid which contains a 1.1 kbp cDNA insert containing among others the presumed gene coding for the deoxynucleoside kinase was obtained from the Berkeley Drosophila genome sequencing project (clone LD15983). The first 600 base pairs at the 5' end of the 1.1 kbp cDNA that were cloned into the multiple cloning site (MCS) of the phagmid using EcoRI and XhoI had already been sequenced by Harvey et al., University of California, Berkeley. On the basis of this sequence information new primers were designed (DM-TK1 and DM-TK2/SEQ ID NO.9 and SEQ ID NO 10) which enabled a complete sequencing of the insert. DM-TK1 is a 21 bp sense primer that binds upstream of the presumed translation start region. DM-TK2 was designed as 21 bp sense primer according to the 3' region of the already sequenced part of the cDNA.

This sequence enabled an open reading frame to be identified comprising 750 bp and coding for a protein with 250 amino acids. The DNA sequence and the amino acid sequence derived therefrom are shown in SEQ ID NO.1 (fig. 8.3/p. 41). The calculated molecular weight of this protein was determined to be 29 kDa and thus corresponds with the data of Munch-Peterson et al. 1998 who have described the size of native DM-dNK to be approximately 30 kDa.

On the basis of the sequence information it was possible to isolate the structural gene coding for DM-dNK from the 1.1 kbp cDNA insert of the pBluescript SK +/- phagmid by means of the "polymerase chain reaction" technique (PCR) (Mullis, K.B. and Faloona, F.A., Methods in Enzymol. 155 (1987) 335-350). A specific primer pair (see SEQ ID NO. 11 and SEQ ID NO.12) with appropriate restriction endonuclease cleavage sites for later insertion into suitable expression plasmids was synthesized for this purpose. Thus, the 5'-primer (DM-dNK3) has an EcoRI restriction endonuclease cleavage site upstream of the coding sequence whereas the 3'-primer (DM-dNK4) contains a HindIII restriction endonuclease cleavage site downstream of the coding sequence. Furthermore, the 3'-primer contains two stop codons downstream of the coding sequence to ensure termination of translation.

Cloning the structural gene for DM-dNK into pUC18

The PCR preparation was applied to an agarose gel and the structural gene of approximately 750 bp in size was isolated from the agarose gel. The PCR fragment was cleaved for 1 hour at 37°C with the EcoRI and HindIII restriction endonucleases. The pUCIS plasmid was simultaneously cleaved for 1 hour at 37°C with the EcoRI and HindIII restriction endonucleases, the preparation was separated by agarose gel electrophoresis and the 2635 bp vector fragment was isolated. Subsequently, the PCR fragment and the vector fragment were ligated together by T4-DNA-ligase. For this 1 µl (20 ng) vector fragment and 3 µl (100 ng) PCR fragment, 1 µl 10 x ligase buffer (Maniatis et al., 1989 B.27), 1 µl T4-DNA-ligase and 4 µl sterile redistilled H_2O were pipetted, carefully mixed and incubated overnight at 16°C.

The cloned gene was checked by means of restriction analysis and by sequencing.

Cloning the structural gene for DM-dNK into suitable expression vectors

In order to express DM-dNK the structural gene was cloned into suitable expression vectors in such a manner that the structural gene is inserted in the correct orientation under the control of a suitable promoter, preferably an inducible promoter and particularly preferably the lac, lacUV5, tac or T5 promoter. Preferred expression vectors are pUC plasmids with lac or lacUVS promoters or pKK plasmids.

For this, the structural gene for DM-dNK was cut out of the plasmid pUC18 using EcoRI and HindIII, the restriction mixture was separated by agarose gel electrophoresis and the approximately 750 bp fragment was isolated from the agarose gel. Simultaneously, the expression vectors were cleaved with EcoRI and HindIII, the restriction mixture was separated by agarose gel electrophoresis and the resulting vector fragment was isolated from the agarose gel. The fragments obtained in this manner were ligated together as described. The correct insertion of the gene was verified by restriction analysis and sequencing.

Transformation of the expression vectors in different E-coli expression strains.

Competent cells of different E. coli strains were prepared according to the method of Hanahan (J. Mol. Biol. 166 (1983) pp. 557). 20 ng isolated plasmid DNA (expression vectors) was added to 200 µl of cells prepared in this manner. After 30 min. incubation on ice they were heat shocked (90 sec. at 42°C). Subsequently, the cells were transferred to 1 ml LB-medium and incubated for 1 hour at 37°C for phenotypical expression. Aliquots of this transformation preparation were plated out on LB plates containing ampicillin as a selection marker and incubated for 15 hours at 37°C.

Suitable host cells are E. coli K12 JM83, JM101, JM105, NM522, UT5600, TG1, RR1ΔM15, E.coli HB101, E.coli B.

Expression of DM-dNK in E.coli

For the expression of DM-dNK clones containing plasmid were inoculated in 3 ml Lb_{amp} medium and incubated in a shaker at 37°C. The cells were induced with 1mM IPTG at an optical density of 0.5 at 550 nm and incubated in a shaker for 4 hours at 37°C. Subsequently, the optical density of the individual expression clones was determined, an aliquot corresponding to an OD_{550} of 3/ml was taken and the cells were centrifuged (10 min., 6000 rpm, 4°C). The cells were resuspended in 400 µl TE buffer (50 mM TRIS/50 mM EDTA, pH 8.0), lysed by ultrasound and the soluble protein fraction was separated from the insoluble protein fraction by centrifugation (10 min., 14000 rpm, 4°C). An application buffer containing SDS and β-mercapto-ethanol was added to all fractions and the proteins were denatured by boiling (5 min. at 100°C). Subsequently 10 µl of each was analyzed by means of a 15 % analytical SDS gel (Laemmli U.K. (1970) *Nature* 227: pp. 555-557).

INFORMATION FOR SEQ ID NO. 9 (DM-dNK1)

SEQUENCE CHARACTERISTICS:
(A) LENGTH : 21 base pairs
(B) TYPE : nucleic acid
(C) STRANDNESS : single
(D) TOPOLOGY : linear

5'-TCCCAATCTCACGTGCAGATC-3'

INFORMATION FOR SEQ ID NO. 10: (DM-dNK2)

SEQUENCE CHARACTERISTICS:
(A) LENGTH : 21 base pairs
(B) TYPE : nucleic acid
(C) STRANDNESS : single
(D) TOPOLOGY : linear

5'-TTCATCGAAGAGTCCATTCAC-3'

INFORMATION FOR SEQ ID NO. 11: (DM-dNK3)

SEQUENCE CHARACTERISTICS:
(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDNESS : single
(D) TOPOLOGY : linear

5'-GCGCGAATTCATGGCGGAGGCAGCATCCTGTGC-3'

INFORMATION FOR SEQ ID NO. 12: (DM-dNK4)

SEQUENCE CHARACTERISTICS:
(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDNESS : single
(D) TOPOLOGY : linear

5'-GCGCAAGCTTATTATCTGGCGACCCTCTGGC-3'

Yet another subject matter of the invention is a method for producing the nucleoside monophosphates which is further characterized by the following steps:

- Synthesis of the nucleoside monophosphates starting from nucleosides by enzymatic phosphorylation using a kinase according to the invention as an enzyme.
- Use of a nucleotide triphosphate as a phosphate group donor in catalytic amounts.
- In situ regeneration of the phosphate group donor by a regenerating system (CK/CP; PK/PEP; acetyl phosphate/acyl kinase, pyrophosphate / pyrophosphorylase).

Nucleoside monophosphates within the sense of the invention are the actual nucleoside monophosphates, deoxynucleoside monophosphates, dideoxynucleoside monophosphates as well as other sugar-modified and base-modified nucleoside monophosphates.

In addition the present invention concerns the use of the kinase according to the invention for nucleoside monophosphate synthesis.

Brief description of the figures:

Fig. 1: The kinetic constants of various nucleoside kinases are shown in fig. 1 (hTK1/2 = human thymidine kinase 1/2 hdCK=human deoxycytidine kinase; hdGK=human deoxyguanosine kinase; HSV= herpes simplex virus). The data are taken from:

Munch Petersen et al. J. Biol. Chem. 266, 9032 (1991); J. Biol. Chem. 268, 15621 (1993),
Biochem. Biophys. Acta 1250, 158 (1995),
Bohmann and Eriksson Biochemistry, 27 4258 (1988),

Wang et al. J. Biol. Chemistry 268, 22847 (1993),
Iwatsuki et al. J. Mol. Biol. 29, 155 (1967),
Black et al. J. Gen. Virology 77, 1521 (1996),
Ma et al. P.N.A.S. 93, 14385 (1996).

Fig. 2: Figure 2 shows the formation of d-CMP from cytidine under the conditions mentioned in example 2.

Fig. 3: Figure 3 shows the formation of d-AMP from adenosine and d-GMP from guanosine under the conditions mentioned in example 4.

Fig. 4: Figure 4 shows the formation of d-CMP from cytidine under the conditions mentioned in example 3.

Fig. 5: Figure 5 shows the DNA sequence of the clone.

Fig. 6: Figure 6 shows the temperature optimum of the nucleoside kinase from D. melanogaster.

Fig. 7: Figure 7 shows the stability of the recombinant Dm-nucleoside kinase compared to isolated Dm-nucleoside kinase. Figure 7A was determined without addition of BSA, fig. 7B with addition of BSA.

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11. Sambrook J., Fritsch E.F., Maniatis T., (1989) *Molecular cloning: A Laboratory Manual second Edition*, B.27 Cold Spring Harbor Laboratory Press NY (USA)
12. Mullis, K.B. und Faloona, F.A., (1987) *Methods in Enzymol.* 155: 335-350
13. Munch-Peterson B., Piskur J. und Sondergaard L. (1998) J. *Biol. Chem.* 273, 3926-3931
14. Laemmli U.K. (1970) *Nature* 227: pp. 555-557

The invention is further elucidated by the following examples:

Example 1:

Comparison of the synthesis of d-CMP with and without thiol addition

d-Cyt	22 mg
Tris buffer pH 8.0	2 ml
MgAc	10 mg
ATP	66 mg
d-NK	0.132 U
CK	100 U
DTT	7 mg/0 mg

The rate of conversion is determined by integrating the peak areas in the HPLC. The conversion is not significantly slower in the mixture without DTT and above all, does not terminate even after 45 hours (see figure 2).

Example 2

Synthesis of d-GMP and d-AMP

d-Ado or d-Guo	28 mg
water	2 ml
MgAc	32 mg
ATP	3 mg
CK	100 U
d-NK	0.396 U

The reaction continues for 32 hours without interruption, the conversion rate is more than 80 % and no thiols have to be added. It is possible to omit the addition of Tris buffer (see figure 3).

Example 3

Synthesis of d-CMP

d-Cyt	22 mg
Tris buffer pH 8.0	2 ml
MgAc	32 mg
ATP	3 mg
CK	100 U
d-NK	0.132 U

The enzyme is still active even after 66 hours despite the absence of thiol stabilizers. The conversion rate is 80 % despite the use of only catalytic amounts of ATP (see figure 4).

Example 4

Synthesis of NMPs, dd-NMPs and base-modified d-NTPs

Substrate solution

CP	250 mg
ATP	7 mg
Mg acetate	160 mg

in 25 ml 50 mM Tris pH 8.0

0.5 ml aliquots of the solution were added to about 2.5 mg of the corresponding nucleoside in each case. Then 50 U creatine kinase and 0.32 U d-NK were added.

Preparation	Nucleoside	Time	Conversion rate
a)	Cytidine	15 h	90 %
b)	dd-adenosine	70 h	40 %
c)	Iso-guanosine	2 h	80 %

Example 5

Activity of the kinase from D. melanogaster at different temperatures

The activity of the Dm-nucleoside kinase was determined at different temperatures. It shows a wide optimum with a maximum at 60°C (see figure 6). The activity test is described in the literature reference No. 14.

Example 6

Activity of recombinant Dm-kinase compared to native Dm-kinase.

The activity of recombinant Dm-kinase compared to native, isolated Dm-kinase was determined. After various periods of incubation in 50 mM Tris pH 7.5 + 2.5 mM $MgCl_2$ at 37°C the remaining activity was determined.

Whereas the recombinant Dm-kinase remains stable without the addition of BSA, the activity of the native kinase decreases within 50 min to < 20 %. The native kinase also remains stable when 2.5 mg/ml BSA is added (fig. 7A + 7B).

The half-life in Tris buffer in the presence of $MgCl_2$ is 50 h, without $MgCl_2$ it is 31 h and in pure water it is 28 h. The native Dm-kinase has a half-life of <12 min. under the same conditions.

Claims

1. Recombinant kinase which is stable during nucleoside monophosphate synthesis without the addition of stabilizing SH reagents and without stabilizing proteins, and which accepts all four natural deoxynucleotides, and is obtainable from insect cells.

2. Recombinant kinase as claimed in claim 1 which, in a purified form, has a specific activity of at least 20 U/mg (1U = 1µmol/min) for all 4 natural deoxynucleotides.

3. Recombinant kinase as claimed in claim 1 or 2 which has a specificity constant k_c/K_m of $> 10000\ M^{-1}\ s^{-1}$ for all natural deoxynucleosides.

4. Recombinant kinase as claimed in one of the claims 1 to 3 which has a wide temperature optimum between 40 and 60°C.

5. Recombinant kinase as claimed in one of the claims 1 to 4 obtainable from Drosophila melanogaster.

6. DNA sequence encoding a kinase from Drosophila melanogaster as claimed in one of the claims 1 to 5.

7. Vector containing the DNA sequence as claimed in claim 6 and a promoter.

8. Host transformed with a vector as claimed in claim 7.

9. Process for the production of a recombinant kinase as claimed in one of the claims 1 to 5, wherein the following steps are carried out:
   1) isolating the coding sequence of DM-dNK,

2) cloning the structural gene into preferred expression vectors for E. coli with inducible promoters,
3) transforming the expression vectors in preferred E. coli host strains and
4) expressing the DM-dNK gene in E. coli by appropriate induction.

10. Use of a recombinant kinase obtainable from Drosophila melanogaster as claimed in one of the claims1 to 5 for nucleoside monophosphate synthesis.

11. Process for the production of a nucleoside monophosphate, wherein a recombinant kinase as claimed in claims 1 to 5 is used to phosphorylate a nucleoside.

Abstract

Recombinant kinase which is stable during nucleoside monophosphate synthesis without the addition of stabilizing SH reagents and without stabilizing proteins and which accepts all four natural deoxynucleotides, and is obtainable from insect cells such as e.g. Drosophila melanogaster. In addition, the invention concerns DNA sequences, vectors, transformed cells, a process for the production of the recombinant kinase as well as its use for producing nucleoside monophosphates.

Figure 1 Kinetic constants of deoxynucleoside kinases

		dThd	dCyd	dAdo	dGuo	ATP
Dm-dNK	k_c (1/s)	15	15	17.8	18.9	15
	K_m (μM)	0.9	1	109	650	1
	k_c/K_m $M^{-1}s^{-1}$	1.7 x 10^7	1.5 x 10^7	1.6 x 10^5	2.9 x 10^4	1.5 x 10^7
hTK1 (1)	k_c (1/s)	4.2				4.2
	K_m (μM)	0.5				140
	k_c/K_m $M^{-1}s^{-1}$	8.3 x 10^6				3 x x 10^4
hTK2 (1)	k_c (1/s)	0.3	0.4			0.3
	K_m (μM)	16	16			2
	k_c/K_m $M^{-1}s^{-1}$	1.9 x 10^4	2.5 x 10^4			1.5 x 10^5
hdCK (2)	k_c (1/s)		0.073	0.31	0.4	0.073
	K_m (μM)		1	120	150	10
	k_c/K_m $M^{-1}s^{-1}$		7.3 x 10^4	2.6 x 10^3	2.7 x 10^3	7.3 x 10^3
hdGK (3)	k_c (1/s)			1.35 x 10^{-3}	2.1 x 10^{-3}	?
	K_m (μM)			60	7.6	?
	k_c/K_m $M^{-1}s^{-1}$			2.3 x 10^1	2.8 x·10^2	?
E. coli TK (4)	k_c (1/s)	1.6				1.6
	K_m (μM)	9				500
	k_c/K_m $M^{-1}s^{-1}$	1.8 x 10^5				3.2 x 10^3
HSV TK (5)	k_c (1/s)	0.21	like dThd (?)			0.21
	K_m (μM)	0.6				32
	k_c/K_m $M^{-1}s^{-1}$	3.8 x 10^5				6.6 x 10^3
Lactobac. dAK/dCK (6) or dGK	k_c (1/s)		0.91	0.05	0.7	0.91
	K_m (μM)		4.5	8.4	7.8	1000
	k_c/K_m $M^{-1}s^{-1}$		2 x 10^5	6 x 10^3	8.9 x 10^4	9.1 x 10^2

Figure 2

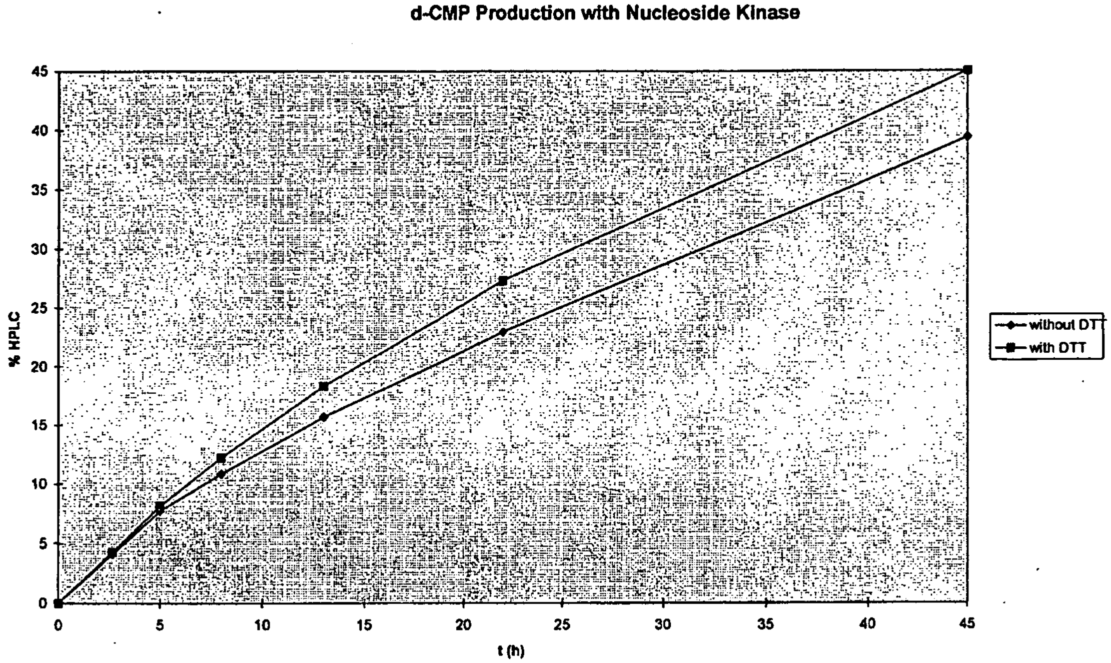
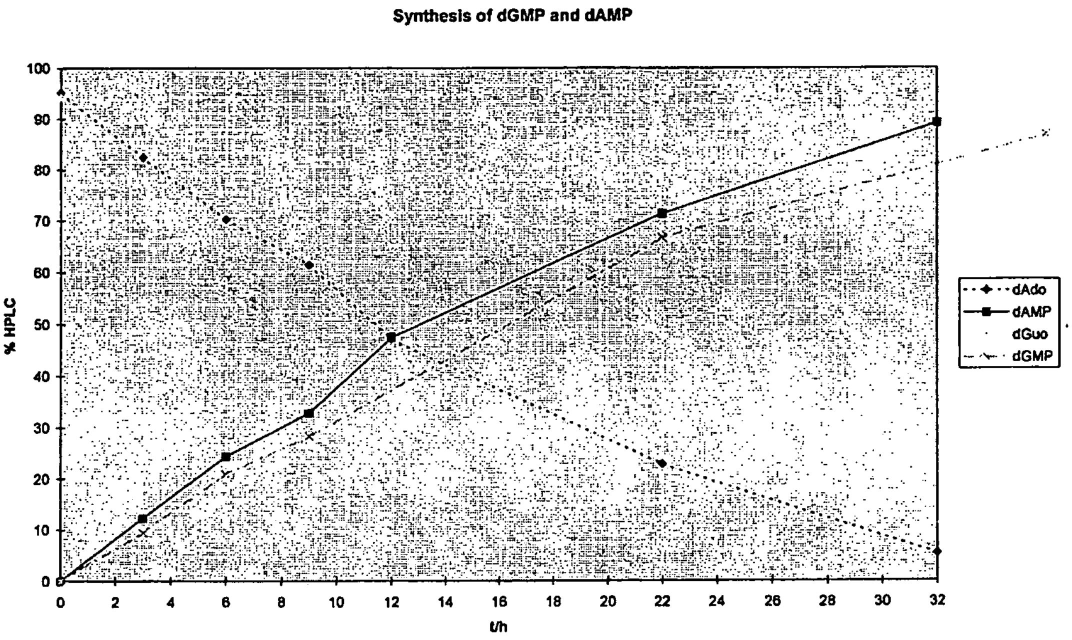

d-CMP Production with Nucleoside Kinase
% HPLC
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
t (h)
without DTT
with DTT

Figure 3


Synthesis of dGMP and dAMP
100
90
80
70
60
50
40
30
20
10
0
% HPLC
0
2
4
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8
10
12
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dAdo
dAMP
dGuo
dGMP

Figure 4

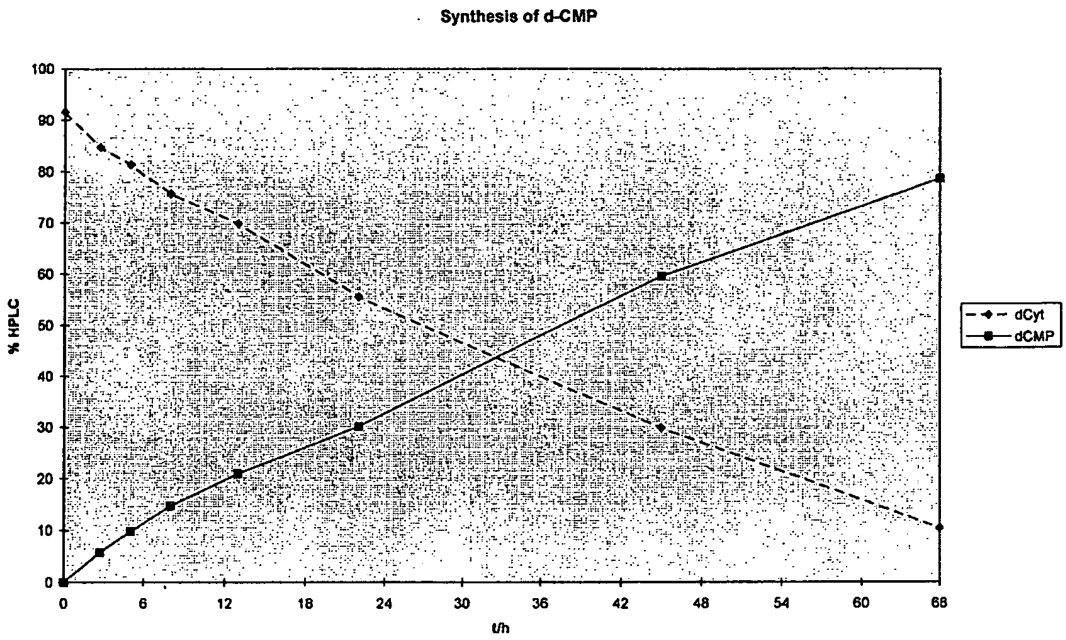

Synthesis of d-CMP
% HPLC
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
54
60
66
t/h
dCyt
dCMP

Figure 5

Seq. ID No.: 1

GAT CTG GTT CCG CGT
GGA TCC ATG GCG GAG GCA GCA TCC TGT GCC CGA AAG GGG ACC AAG TAC GCC
GAG GGC ACC CAG CCC TTC ACC GTC CTC ATC GAG GGC AAC ATC GGC AGC GGG
AAG ACC ACG TAT TTG AAC CAC TTC GAG AAG TAC AAG AAC GAC ATT TGC CTG
CTG ACC GAG CCC GTC GAG AAG TGG CGC AAC GTC AAC GGG GTA AAT CTG CTG
GAG CTG ATG TAC AAA GAT CCC AAG AAG TGG GCC ATG CCC TTT CAG AGT TAT
GTC ACG CTG ACC ATG CTG CAG TCG CAC ACC GCC CCA ACC AAC AAG AAG CTA
AAA ATA ARG GAG CGC TCC ATT TTT AGC GCT CGC TAT TGC TTC GTG GAG AAC
ATG CGA CGA AAC GGC TCG CTG GAG CAG GGC ATG TAC AAT ACG CTG GAG GAG
TGG TAC AAG TTC ATC GAA GAG TCC ATT CAC CTG CAG GCG GAC CTC ATC ATA
TAT CTG CGC ACC TCG CCG GAG GTG GCG TAC GAA CGC ATC CGG CAG CGG GCT
CGT TCT GAG GAG AGC TGC GTG CCG CTT AAG TAC CTT CAG GAG CTG CAT GAG
TTG CAC CAG GAC TGG TTG ATA CAC CAG AGA CGA CCG CAG TCG TGC AAG GTC
CTA GTC CTC GAT GCC GAT CTG AAC CTG GAA AAC ATT GGC ACC GAG TAC CAG
CGC TCG GAG AGC AGC ATA TTC GAC GCC ATC TCA AGT AAC CAA CAG CCC TCG
CCG GTT CGT GTG TCG CCC AGC AAG CGC CAG AGG GTC GCC AGA TAA GGA ATT
CAT CGT GAC TGA

Figure 6

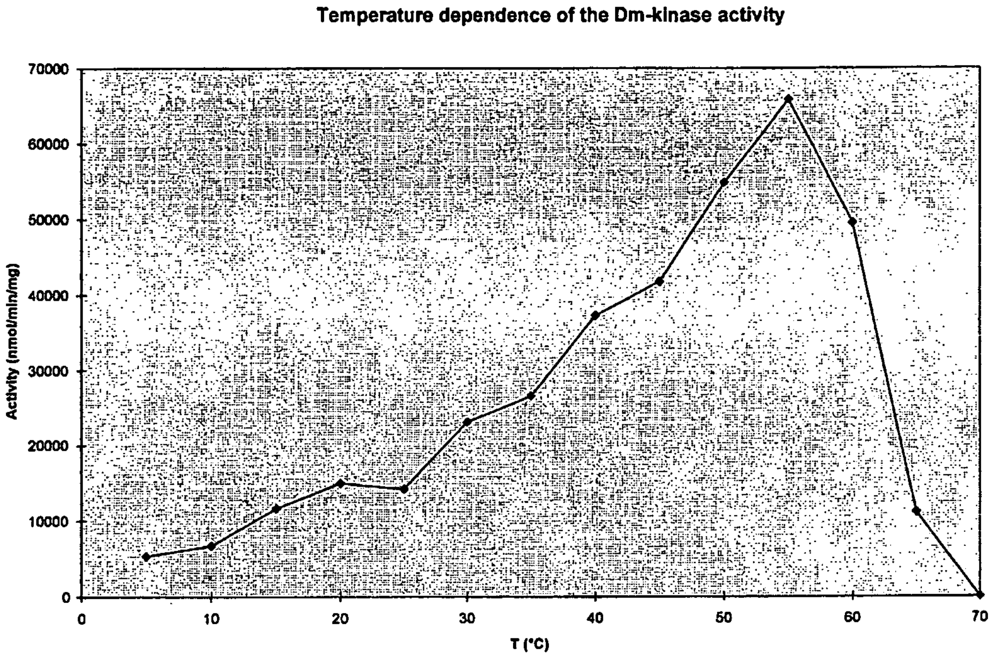

Temperature dependence of the Dm-kinase activity
Activity (nmol/min/mg)
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
70
T (°C)

Figure 7A

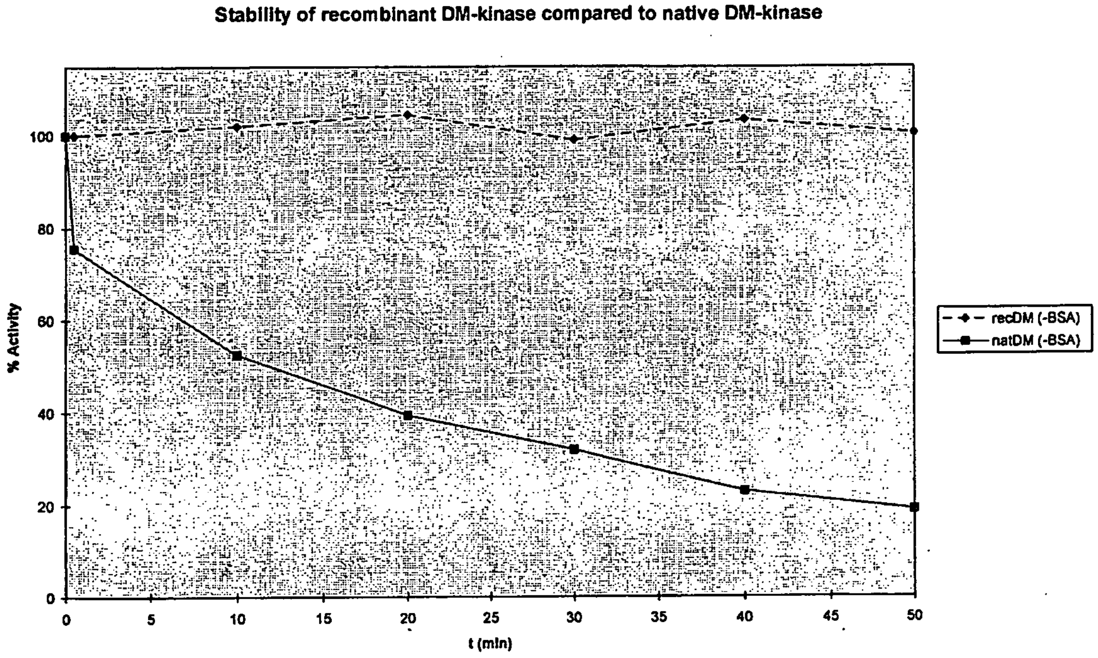
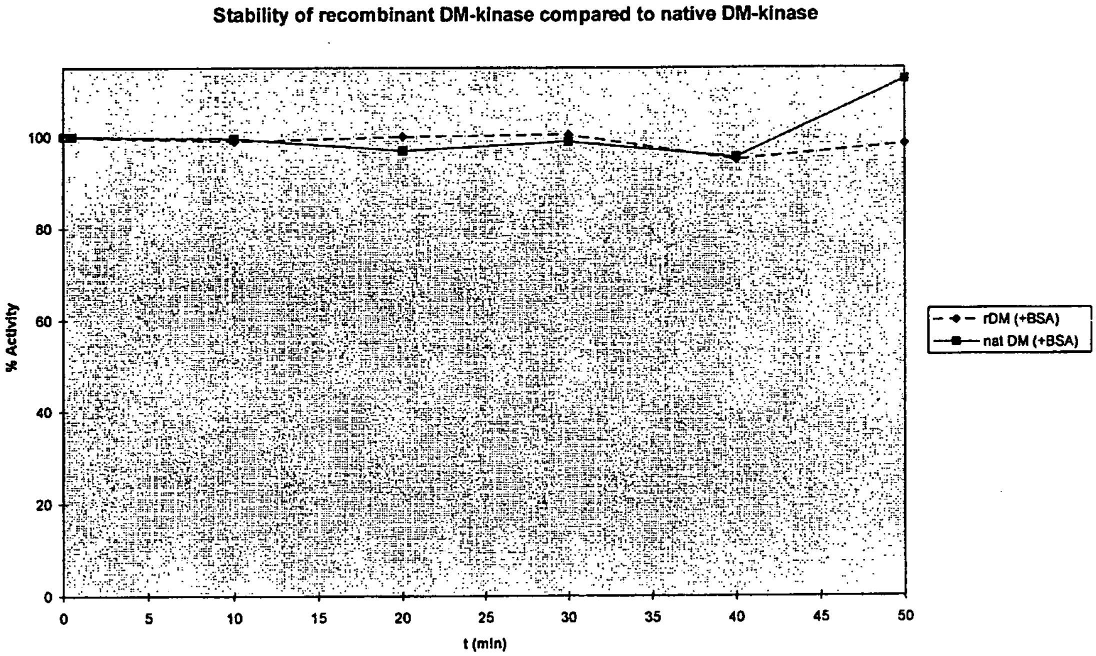

Stability of recombinant DM-kinase compared to native DM-kinase
% Activity
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
t (min)
recDM (-BSA)
natDM (-BSA)

Figure 7B


Stability of recombinant DM-kinase compared to native DM-kinase
% Activity
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
t (min)
rDM (+BSA)
nat DM (+BSA)

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